

## Kligler Iron HiVeg<sup>®</sup> Agar

MV078

### Intended Use:

Recommended for differential identification of gram-negative enteric bacilli from various samples on the basis of the fermentation of glucose (dextrose), lactose and hydrogen sulphide production.

### Composition\*\*

Ingredients	g / L
HiVeg <sup>®</sup> special peptone	15.000
HiVeg <sup>®</sup> extract	3.000
Yeast extract	3.000
HiVeg <sup>®</sup> peptone No. 3	5.000
Lactose	10.000
Dextrose (Glucose)	1.000
Ferrous sulphate	0.200
Sodium chloride	5.000
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	15.000
Final pH ( at 25°C)	7.4±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 57.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool to 45-50°C in slanted position to form slopes with about 1 inch butts. Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

Note: Avoid overheating otherwise it may produce precipitate in the medium.

### Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (1,2) and Russels Double Sugar Agar (3) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (4). Bailey and Lacey substituted phenol red for Andrade indicator previously used as pH indicator (4). Kligler Iron HiVeg<sup>®</sup> Agar is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associated with animal peptones. Kligler Iron HiVeg<sup>®</sup> Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella* Typhi from other *Salmonellae* and also *Salmonella* Paratyphi A from *Salmonella* Scottmuelleri and *Salmonella* Enteritidis (5). Fermentation of dextrose results in production of acid, which turns the indicator from red to yellow. Since there is little sugar i.e. dextrose, acid production is very limited and therefore a reoxidation of the indicator is produced on the surface of the medium, and the indicator remains red. However, when lactose is fermented, the large amount of acid produced, avoids reoxidation and therefore the entire medium turns yellow. Kligler Iron HiVeg<sup>®</sup> Agar, in addition to HiVeg<sup>®</sup> special peptone, HiVeg<sup>®</sup> extract, HiVeg<sup>®</sup> peptone No. 3 and yeast extract, contains lactose and glucose (dextrose), which enables the differentiation of species of enteric bacilli. Phenol red is the pH indicator, which exhibits a colour change in response to acid produced during the fermentation of sugars. The combination of ferrous sulphate and sodium thiosulphate enables the detection of hydrogen sulfide production, which is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Lactose non-fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid produced by the fermentation of the small amount of glucose (dextrose). When glucose (dextrose) supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids produced. The reversion does not occur in the anaerobic environment of the butt, which therefore remains acidic (yellow butt). Lactose fermenters produce yellow slants and butts because of lactose fermentation. The high amount of acids thus produced helps to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the medium indicates the fermentation of neither glucose (dextrose) nor lactose. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar by the formation of cracks in the butt of the medium.

Pure cultures of suspected organisms from plating media such as MacConkey HiVeg® Agar (MV081), Bismuth Sulphite HiVeg® Agar (MV027) or Deoxycholate Citrate Agar, HiVeg® (MV065); SS HiVeg® Agar (MV108) etc. are inoculated on Kligler Iron HiVeg® Agar for identification.

## Type of specimen

Isolated Microorganism from food, dairy and water samples.

## Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(6).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9).

After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1. Results should be noted after 18-24 hours. Else it might result in erroneous results.
2. Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous results.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

### Appearance

Light yellow to pink homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in tubes as slants

### Reaction

Reaction of 5.75% w/v aqueous solution at 25°C. pH : 7.4±0.2

### pH

7.20-7.60

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18 - 48 hours.

Organism	Growth	Gas	H <sub>2</sub> S	Slant	Butt
<i>Escherichia coli</i> ATCC 25922 (00013*)	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
<i>Citrobacter freundii</i> ATCC 8090	luxuriant	positive reaction	positive reaction, blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

## <i>Proteus hauseri</i> ATCC 13315	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
<i>Klebsiella pneumoniae</i> ATCC 13883 (00087*)	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
<i>Salmonella Paratyphi A</i> ATCC 9150	luxuriant	positive reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
<i>Salmonella Schottmuelleri</i> ATCC 10719	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
<i>Salmonella Typhi</i> ATCC 6539	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
<i>Salmonella Enteritidis</i> ATCC 13076 (00030*)	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
<i>Shigella flexneri</i> ATCC 12022 (00126*)	luxuriant	negative reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	luxuriant	negative reaction	negative reaction, blackening of medium	alkaline reaction, red colour of the medium	alkaline reaction,red colour of the medium
<i>Yersinia enterocolitica</i> ATCC 27729	luxuriant	variable reaction	negative reaction,no blackening of medium	alkaline reaction,red colour of the medium	acidic reaction, yellowing of the medium
<i>Enterobacter cloacae</i> ATCC 13047 (00083*)	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

Key :\* Corresponding WDCM numbers

(#) Formerly known as *Enterobacter aerogenes*

## Formerly known as *Proteus vulgaris*

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

## Reference

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